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GENETIC DIVERSITY AND POPULATION STRUCTURE IN THE RARE, ENDEMIC BAKER CYPRESS (*HESPEROCYPARIS BAKERI*)

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ABSTRACT

Baker cypress (*Hesperocyparis bakeri* [Jeps.] Bartel) is one of 10 species of cypress found in western North America. It is restricted to a small number of highly disjunct, isolated populations, making it particularly vulnerable to the influences of genetic drift, inbreeding, and reduced gene flow. Baker cypress is fire adapted and the serotinous cones require heat to open and release seeds. Altered fire regimes have negatively impacted the health and vigor of some populations and lower levels of genetic diversity could make this species more susceptible to the impacts of predicted future climate change. Previously, no information on genetic diversity and population structure of Baker cypress was available. We used 12 polymorphic allozyme loci to assess genetic diversity and population structure for eight of the 11 known populations of Baker cypress. Overall mean observed heterozygosity (H_o) was 0.178 and expected heterozygosity (H_e) was 0.204, values higher than for other cypress species and other fire adapted conifers. Although genetic diversity was relatively high, many populations had a deficiency of heterozygotes (fixation index > 0), most likely due to inbreeding and possibly a Wahlund effect. Population differentiation among seven of the eight populations (northernmost population excluded) was 9%, considerably lower than for other conifers with disjunct populations. Our results indicate that the current population structure of the species is likely a fairly recent reduction from a formerly widespread distribution with differentiation among populations resulting from genetic drift. Implications of genetic diversity and population structure for potential restoration work are discussed.

Key Words: Baker cypress, endemism, fire adaptation, genetic diversity, *Hesperocyparis bakeri*, population structure, restoration, serotiny.

Small, isolated populations within a species often have lower levels of gene flow, reducing the exchange of genetic material. Gene flow can help a species adapt to changes in their environment, but the introduction of new alleles can also swamp alleles in the target population that are locally adapted (Frankham et al. 2002). Populations of long-lived plants such as forest trees have to be able to adapt to their local environment to survive both annual variations in temperature and rainfall as well as long-term changes in climate. In order to track climatic changes during glacial and interglacial periods during the Quaternary period (roughly 1.8 million yr bp to present), tree species have experienced large-scale range shifts, population contractions and expansions, and aggregation and disassociation of plant communities (Davis and Shaw 2001, Petit et al. 2008). More recently, land-use changes have dramatically altered the landscape, especially in mixed-conifer forests, increasing both the density and dominance of shade tolerant species (Merschel et al. 2014). These shade-tolerant species have benefited from these changes, but this often comes at the expense to other species, especially fire dependent species such as Oregon white oak (*Quercus garryana* Douglas ex Hook.) (Devine and Harrington 2006), whitebark pine (*Pinus albicaulis* Engelm.) (Arno and Hoff 1989), and Baker cypress (*Hesperocyparis bakeri* [Jeps.] Bartel) (Merriam and Rentz 2010). In addition, climate change has altered temperature and rainfall patterns (IPCC 2014) and this is occurring at a pace that exceeds the abilities of many forest tree species to migrate in response to these changes (Aitken et al. 2008).

There are ten *Hesperocyparis* species native to California, eight of which are endemic (Baldwin et al. 2012) and all are comprised of relatively small, disjunct and isolated populations. The majority of these species have little or no published information on genetic diversity (heterozygosity) and population structure. Species with small, shrinking, or isolated populations may be genetically vulnerable because they often are prone to the influences of genetic drift, genetic bottlenecks, and higher levels of inbreeding than more widespread species with continuous population distributions. It should also be noted that there has been taxonomic instability of *Cupressus* L. in the last decade (Terry 2012), with the introduction of the genus *Xanthocyparis* by Farjon (2002) and the movement of western hemisphere cypresses (originally *Cupressus*) first to the genus *Callotropsis* (Little 2006), then to *Hesperocyparis* (Adams et al. 2009). *Hesperocyparis* is currently used by the Jepson eFlora (Jepson Flora Project 2017) and the USDA Plants database (USDA NRCS 2017) for

TABLE 1. Site names and characteristics for eight Baker cypress populations sampled for genetic analysis (from Merriam and Rentz 2010).

Site no.	Site name	Latitude	Longitude	Est. size (ha)	Elevation (m)	Avg. tree age (range)	Soil type
1	Goosenest	41.7396	−122.2583	120	1500–1850	98 (53–163)	Volcanic
2	Flounce Rock	42.7343	−122.6036	1.2	1230	132 (108–174)	Metasedimentary
3	Miller Lake	42.0677	−123.2957	20	1230–1600	N/A	Metasedimentary
4	Timbered Crater	41.2271	−121.4481	2800	1070–1230	70 (25–125)	Volcanic
5	Wheeler Peak	40.1032	−120.7034	30	1970–2120	95 (46–158)	Volcanic
6	Burney	40.7713	−121.6097	600	1385–1540	56 (2–96)	Volcanic
7	Seiad	41.9145	−123.1487	320	925–1170	68 (32–113)	Ultramafic
8	Hamburg	41.7646	−123.1063	6.4	1350–1600	149 (116–196)	Ultramafic

North American cypresses and is what we have used here.

Baker cypress (*Hesperocyparis bakeri*) is a rare endemic currently found in eleven widely scattered and isolated populations in northern California and southwestern Oregon. In the Siskiyou Mountains it occurs on serpentine and granitic soils, while in the Cascades and Sierra Nevada it occurs on volcanic soils (Esser 1994). Baker cypress is unique in that its range includes the northernmost (Flounce Rock) (Callahan 2013), as well as the highest elevation (Wheeler Peak) population of cypress in North America (Keeler-Wolf 1985); the Wheeler Peak population is the furthest population of cypress from the coast in California (Keeler-Wolf 1985).

Baker cypress is relatively long lived and is wind pollinated; life history traits often associated with high genetic diversity and population connectivity, and lower interpopulation differentiation (Hamrick and Godt 1989, Hamrick et al. 1992, Hamrick and Godt 1996). However, it also is an early seral, endemic species with a very limited distribution, serotinous cones, and gravity-dispersed seed, life history traits that would be consistent with lower genetic diversity, higher inbreeding and significant interpopulation genetic subdivision (Loveless and Hamrick 1984). Due to the small size of many of the extant populations, and the cumulative impacts of fire exclusion, there is a need for restoration in some Baker cypress populations. However, while Baker cypress has been included in phylogenetic analyses of western hemisphere cypresses (Bartel et al. 2003, Little 2006, Mao et al. 2010, Terry et al. 2012), no information on genetic diversity and population structure of the species has been available to date, which is important for any restoration treatments to ensure that, at the minimum, genetic diversity is not lost.

Population geographic range sizes in the eleven known stands of Baker cypress range from <1.2 to over 2800 hectares; eight of these populations are less than 140 hectares, with three populations being less than 8 hectares (Merriam and Rentz 2010) (Table 1). Some of the smallest populations are in the poorest condition and in need of restoration (Merriam and Rentz 2010). Merriam and Rentz (2010) documented stand health by assessing crown condition, stand age structure and density, fuel loading, cones per tree and viable seeds per cone, and presence of regeneration.

Baker cypress is fire adapted and stand replacing fires generally produce conditions favorable to regeneration by removing duff and heating the cones so that seeds are released. In several populations, fire exclusion is leading to the replacement of Baker cypress by shade tolerant species, senescence, and generally poor stand conditions. One of these populations that was in very poor condition, Mud Lake (not sampled in this study), burned in the 2007 Moonlight Fire and substantial post-fire regeneration of Baker cypress has been observed (Merriam and Rentz 2010).

The objective of this study was to assess genetic diversity and population differentiation across the range of the species to determine 1) the level of genetic diversity present within and among populations, and 2) if geographically isolated populations are genetically distinct to aid in the development of management plans for restoration or population conservation of this species.

METHODS AND MATERIALS

Tissue Collection

Branch tips with current-year's foliage were collected from approximately 30 individuals per population for eight of the 11 populations that comprise the known distribution of Baker cypress (Fig. 1, Table 1). Samples from most sites were collected in June 2012 with the exception of the Seiad stand, which was collected in September 2012. Individual tree samples were placed in sealed plastic bags and stored in a cooler with ice packs or refrigerator until delivered to the USDA Forest Service National Forest Genetics Laboratory (NFGEL) in Placerville, CA for processing and genetic marker analysis. Samples were delivered to NFGEL within 6 d of collection.

Allozyme Analysis

Allozyme markers were utilized for this study, as they are a simple, inexpensive, and efficient method for assessing genetic diversity and population structure. In addition, the numerous studies of conifers

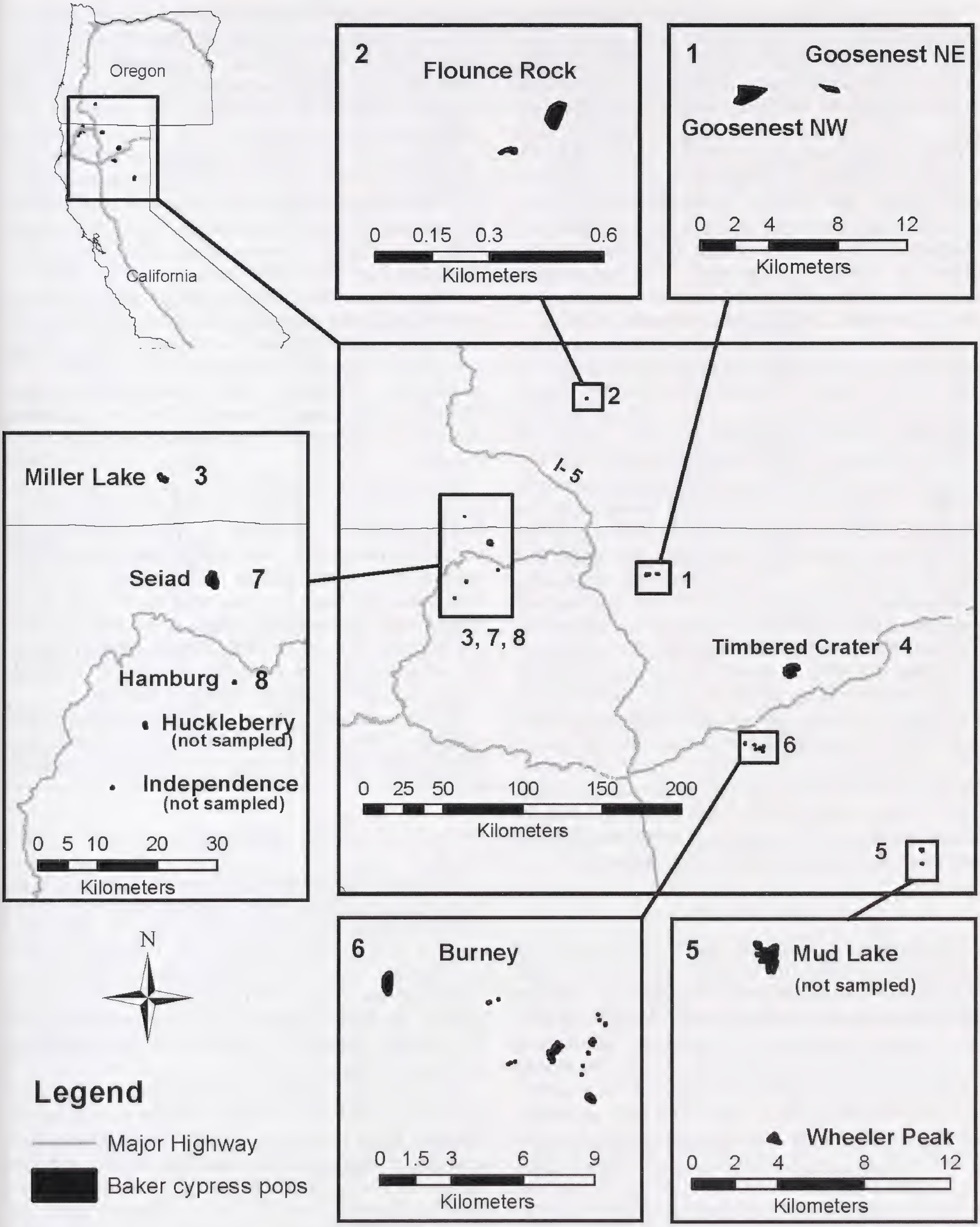

FIG. 1. Species distribution of Baker cypress, including unsampled populations. Numbers refer to populations in Table 1.

using allozymes (Conkle 1987, Hamrick and Godt 1996, Truesdale and McClenaghan 1998, Ledig et al. 2005) allows for a direct comparison with other related species or species with similar distributions or ecological strategies. At the time of this work, DNA based markers were not available for analysis of this species; allozymes, while maybe less informative than some markers available now, were used as they would yield sufficient data to address the questions of interest.

Approximately 100 mg of leaf tissue per tree was ground to fine powder in mortars with pestles under liquid nitrogen. A total of 0.5 ml of a 0.1 M Tris-HCl (pH 8.0) extraction buffer, with 10% (w/v) polyvinylpyrrolidone-40, 10% sucrose, 0.17% EDTA (Na2 salt), 0.15% dithiothreitol, 0.02% ascorbic acid, 0.10% bovine albumin, 0.05% NAD, 0.035% NADP, and 0.005% pyridoxal-5-phosphate (Pitel and Cheliak 1984, USDA Forest Service 2012) was added to the powder to create a slurry. Slurry was transferred to microtiter plates and samples were frozen at –70°C. On the day of electrophoresis, samples were thawed and the extracts were absorbed onto 3 mm wide chromatography paper wicks.

Methods of sample preparation and electrophoresis follow the general methodology of Conkle et al. (1982), with some modifications (USDA Forest Service 2012). All enzymes were resolved on 11% starch gels. A lithium borate electrode buffer (pH 8.3) was used with a Tris citrate gel buffer (pH 8.3) (Conkle et al. 1982) to resolve phosphoglucomutase (PGM), malic enzyme (ME), and phosphoglucose isomerase (PGI). A sodium borate electrode buffer (pH 8.0) was used with a Tris citrate gel buffer (pH 8.8) (Conkle et al. 1982) to resolve glutamate-oxaloacetate transaminase (GOT), triose-phosphate isomerase (TPI), glucose-6-phosphate dehydrogenase (G6PD), and uridine diphosphoglucose pyrophosphorylase (UGPP). A morpholine citrate electrode and gel buffer (pH 6.1) (USDA Forest Service 2012) was used to resolve phosphogluconate dehydrogenase (6PGD), malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), and shikimate dehydrogenase (SKD). Enzyme stain recipes follow USDA Forest Service (2012). For quality control, all individuals were run and scored twice. In addition, gels were photographed for future reference.

Data Analysis

Genetic diversity of the eight populations was assessed using GenAlEx (Peakall and Smouse 2006, Peakall and Smouse 2012) to calculate the population mean numbers of alleles, observed and expected heterozygosity, and the fixation index (inbreeding coefficient, measured as F) and their associated standard errors. GenAlEx calculates $F = 1 - (H_o/H_e)$, and Wright's (1965) F-statistics were estimated per allele using mean heterozygosities across populations as $F_{is} = 1 - (H_o/H_e)/H_e$; $F_{it} = 1 - (H_o/H_t)$; $F_{st} = 1 - (H_e/H_t)$, where H_t is the total expected heterozygosity. Genetic differentiation among populations was assessed from pairwise F_{st} values and an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) also using GenAlEx. A Bayesian model-based analysis was used to infer population genetic structure using STRUCTURE version 2.3.3 (Pritchard et al. 2000). Data were analyzed using a no admixture ancestry model as these are fully discrete populations and this model is often more powerful at detecting subtle structure (Pritchard 2010). In this model, correlated allele frequencies were used to estimate the posterior probabilities L (K) of K groups and the individual percentages of group membership assigned according to their multilocus profiles (Falush et al. 2007). We assessed the probabilities for a range of K (K = 1 – 8) using a burn-in period of 10,000 iterations of the Markov chain Monte Carlo model, followed by a run length of 500,000 iterations with the analysis of each level of K repeated 10 times. We calculated Delta K up to K = 8 according to Evanno et al. (2005) and used Structure Harvester (Earl and von Holdt 2012) to visualize the results. The correlation between mean population linear geographic distance and both mean linearized genetic distance and linearized F_{st} among populations was assessed using a Mantel (1967) test in GenAlEx. GenAlEx uses a modification of the Haversine Formula (Sinnott 1984) to calculate distances in kilometers from latitude/longitude coordinates.

The program BOTTLENECK (Cornuet and Luikart 1997) was used to test whether populations had undergone a restriction in effective population size in the recent past. The infinite alleles model was used as it is recommended for allozyme data. The Wilcoxon sign-rank test was used as it is the most appropriate and powerful when fewer than 20 loci are available (Piry et al. 1999). The minimum number of polymorphic loci available was five at Flounce Rock, and the maximum was 11 at Burney.

The correlation of H_o, H_e, F with average tree age and stand size, as reported by Merriam and Rentz (2010) was calculated using SAS software, version 9.3.

RESULTS

Of the 14 loci examined, 12 were polymorphic in at least one population (Table 2). PGI1 and GOT2 were monomorphic, while GOT1 had just one heterozygote in each of the Burney and Timbered Crater populations. There were only two private alleles, with a rare private allele for TPI with just two heterozygotes in the Burney population, and a private allele at a moderate frequency for MDH1 in the Hamburg population (Table 2).

Overall mean observed heterozygosity (H_o) was 0.178 and population mean H_o ranged from 0.100 at Flounce Rock to 0.224 at the Hamburg population, and mean expected heterozygosity (H_e) was 0.204 (range 0.104–0.260) (Table 3). The percent polymorphic loci varied from 35.7% at Flounce Rock to 78.6% at the Burney population, and effective number of alleles varied from 1.36 at Flounce Rock to 2.14 at Burney (Table 3). For all populations except Wheeler Peak, expected heterozygosity exceeded observed heterozygosity resulting in positive values of F (mean F was 0.099) (Table 3). The mean Wright's F_{is} (0.070) was similar to the value of F (Table 4). The value of F for Wheeler Peak was only very slightly negative (–0.043) but not significantly

TABLE 2. Allele frequencies for 14 allozyme loci from eight Baker cypress populations.

Locus	Allele	Goosenest	Flounce Rock	Miller Lake	Timbered Crater	Wheeler	Burney	Seiad	Hamburg
ME	1	0.783	0.883	0.406	0.85	0.833	0.667	0.593	0.726
	2	0.15	0.117	0.5	0.133	0.117	0.333	0.37	0.274
	3	0.067	–	0.094	0.017	0.05	–	0.037	–
PGM	1	0.667	1	0.643	0.65	0.783	0.5	0.648	0.694
	2	0.233	–	0.214	0.2	0.217	0.232	0.278	0.145
	3	0.1	–	0.143	0.15	–	0.268	0.074	0.161
PGI1	1	1	1	1	1	1	1	1	1
PGI2	1	0.517	1	0.771	0.767	0.683	0.533	0.722	0.516
	2	0.483	–	0.229	0.133	0.317	0.433	0.222	0.484
	3	–	–	–	0.1	–	–	0.037	–
	4	–	–	–	–	–	0.033	0.019	–
TPI	1	1	1	1	1	1	0.967	1	1
	2	–	–	–	–	–	0.033	–	–
GOT1	1	1	1	1	0.983	1	0.983	1	1
	2	–	–	–	0.017	–	0.017	–	–
GOT2	1	1	1	1	1	1	1	1	1
G6PD	1	0.55	0.75	0.5	0.65	0.8	0.733	0.556	0.887
	2	0.45	0.25	0.5	0.35	0.2	0.267	0.444	0.113
UGPP	1	0.967	1	1	1	0.933	0.867	0.907	0.919
	2	–	–	–	–	0.017	0.033	–	–
	3	0.033	–	–	–	0.033	0.083	0.074	0.081
	4	–	–	–	–	0.017	0.017	0.019	–
6PGD	1	0.683	1	0.171	0.367	0.117	0.533	0.13	0.548
	2	0.317	–	0.829	0.467	0.883	0.467	0.722	0.419
	3	–	–	–	0.167	–	–	0.148	0.032
SKD1	1	0.233	0.483	0.257	0.117	0.2	0.15	0.148	0.484
	2	0.633	0.517	0.429	0.567	0.8	0.75	0.741	0.419
	3	0.133	–	0.314	0.317	–	0.1	0.111	0.097
MDH1	1	1	1	1	1	1	1	1	0.613
	2	–	–	–	–	–	–	–	0.387
MDH2	1	0.783	0.933	0.914	1	1	0.817	1	0.774
	2	0.217	0.067	0.086	–	–	0.183	–	0.226
IDH	1	1	0.15	0.971	1	1	0.967	1	1
	2	–	0.85	0.029	–	–	0.033	–	–

different from zero (SE = 0.040). Genetic diversity in the Flounce Rock population was lower for all statistics, with the exception of fixation index (F). Excluding the Flounce Rock population mean values were: $H_o = 0.189$, $H_e = 0.218$, $F = 0.106$.

The AMOVA showed that 18% of the observed genetic variation resided among populations while the remaining 82% was among individuals within populations. Overall Wright's F_{st} was 0.171 (SE = 0.058) (Table 4), but excluding Flounce Rock the overall F_{st} was reduced by nearly one half (F_{st} = 0.089. SE = 0.027). The common and rare alleles for the IDH locus in the Flounce Rock population are opposite to the other populations (Table 2), leading to very large values of F_{it} and F_{st} (Table 4). To assess the impact of this locus on overall F_{st}, the analysis was repeated excluding the IDH locus. With IDH excluded overall F_{st} decreased to 0.117 (SE = 0.031), which is similar to the value for all loci excluding the Flounce Rock population indicating that this locus contributed significantly to the genetic differentiation of Flounce Rock from the remaining populations.

TABLE 3. Sample size and mean effective number of alleles (N_e), percent polymorphic loci (%P), observed heterzygosity (H_o), expected heterozygosity (H_e), and fixation index (F) for 14 allozyme loci. Standard errors in parentheses.

Site	N	A (SE)	%P	H_o (SE)	H_e (SE)	F
Goosenest	30	1.79 (0.21)	57.10%	0.190 (0.055)	0.229 (0.063)	0.143
Flounce Rock	30	1.36 (0.13)	35.70%	0.100 (0.042)	0.104 (0.045)	0.02
Miller Lake	35	1.79 (0.21)	57.10%	0.175 (0.053)	0.221 (0.067)	0.136
Timbered Crater	30	1.86 (0.25)	50.00%	0.186 (0.064)	0.202 (0.067)	0.091
Wheeler Peak	30	1.71 (0.24)	50.00%	0.150 (0.046)	0.145 (0.044)	−0.043
Burney	30	2.14 (0.23)	78.60%	0.222 (0.054)	0.257 (0.060)	0.11
Seiad	27	2.00 (0.30)	50.00%	0.180 (0.055)	0.211 (0.062)	0.159
Hamburg	31	1.86 (0.21)	64.30%	0.224 (0.059)	0.260 (0.062)	0.131
Grand Mean		1.81 (0.08)	55.40%	0.178 (0.019)	0.204 (0.021)	0.099

TABLE 4. Estimates of Wright's (1965) F-statistics for 12 polymorphic loci in Baker cypress.

Locus	F_{is}	F_{it}	F_{st}
ME	0.267	0.339	0.098
PGM	0.209	0.263	0.068
PGI2	0.057	0.168	0.118
TPI	−0.034	−0.004	0.029
GOT1	−0.017	−0.004	0.013
G6PD	0.03	0.103	0.075
UGPP	0.28	0.306	0.037
6pgd	0.062	0.345	0.303
SKD1	0.177	0.253	0.093
MDH1	−0.088	0.299	0.356
MDH2	0.032	0.125	0.096
IDH	−0.13	0.738	0.768
Mean	0.070 (0.036)	0.244 (0.053)	0.171 (0.058)

Pairwise F_{st} values among populations ranged from 0.025 to 0.251 (Table 5), although excluding the Flounce Rock population, the highest value was only 0.091 between the Hamburg and Seiad populations. The mean pairwise F_{st} value between Flounce Rock and the other seven populations was 0.172, while the mean among all the other populations was 0.051. The second highest pairwise F_{st} value (0.222) was between Flounce Rock (the northernmost population) and Wheeler Peak (the southernmost population). Unexpectedly, the greatest pairwise F_{st} value was between Flounce Rock and Miller Lake, the geographically closest population to Flounce Rock (Fig. 1). The three Siskiyou populations (Miller Lake, Seiad, and Hamburg) are geographically close and while the pairwise F_{st} value between Miller Lake and Seiad was one of the smallest among all pairwise comparisons (0.025), the values between Hamburg and Seiad (0.091) and Miller Lake (0.089) were among the largest (Table 5). When the IDH locus was excluded, pairwise F_{st} values between Flounce Rock and all other populations were still higher, often by an order of magnitude, than among the remaining populations.

Analysis of genetic structure using the programs STRUCTURE and Structure Harvester revealed five population groups (greatest mean of estimated ln (K) and Delta K at K = 5) (Figs. 2, 3a and b). The STRUCTURE plot (Fig. 2) clearly shows that population 2 (Flounce Rock) was differentiated from all other populations and comprised one of the five groups, as was also clear from the pairwise F_{st} values (Table 4). Figure 2 also shows that population 8 (Hamburg) comprised another group. The remaining populations: 1, 3, 4, 5, 6 and 7 (Goosenest, Miller Lake, Timbered Crater, Wheeler Peak, Seiad, and Burney) comprised the three remaining groups with approximately equal contributions of all three groups in all of these populations. Interestingly, Miller Lake, Seiad, and Hamburg are geographically closest, yet Hamburg did not cluster genetically with these populations. The correlation of both genetic distance and F_{st} with geographic distance tested with the Mantel test did not show a significant relationship between genetic and geographic distance indicating that there does not appear to be isolation by distance among the populations sampled.

The BOTTLENECK analysis indicates that there is evidence of recent reductions in effective population size at half of the populations sampled (Table 6). This Wilcoxon sign-rank test gives the probability of observing expected heterozygosity under the null hypothesis (mutation-drift equilibrium) that is different from the probability under the alternative hypothesis (a bottleneck). An unexpected result is that there does not seem to be a relationship of this test statistic with population geographic size. The significance of the test statistic for Flounce Rock, the smallest population, approached significance ($P = 0.089$), while Timbered Crater, the largest population was marginally significant ($P = 0.055$). The populations with the strongest evidence of having experienced a bottleneck were Hamburg (6.4 hectares), Miller Lake (20 hectares), and Goosenest (120 hectares).

Genetic diversity statistics (H_o, H_e, F) were not related to latitude or longitude (data not shown). All three variables showed a negative relationship with average tree age and a positive relationship with population geographic size, however, the correlations were not significant.

DISCUSSION

Genetic diversity in almost all populations of Baker cypress included in the study was relatively high, with some evidence of past bottlenecks and inbreeding. In addition, with the exception of the small Flounce Rock population, there was moderate to low levels of population differentiation.

TABLE 5. Pairwise population F_{st} values.

	Goosenest	Flounce Rock	Miller Lake	Timbered Crater	Wheeler Peak	Burney	Seiad
Flounce Rock	0.174	-					
Miller Lake	0.062	0.251	-				
Timbered Crater	0.039	0.165	0.043	-			
Wheeler Peak	0.076	0.222	0.055	0.042	-		
Burney	0.018	0.159	0.044	0.037	0.046	-	
Seiad	0.059	0.215	0.025	0.03	0.031	0.034	-
Hamburg	0.053	0.187	0.086	0.073	0.089	0.039	0.091

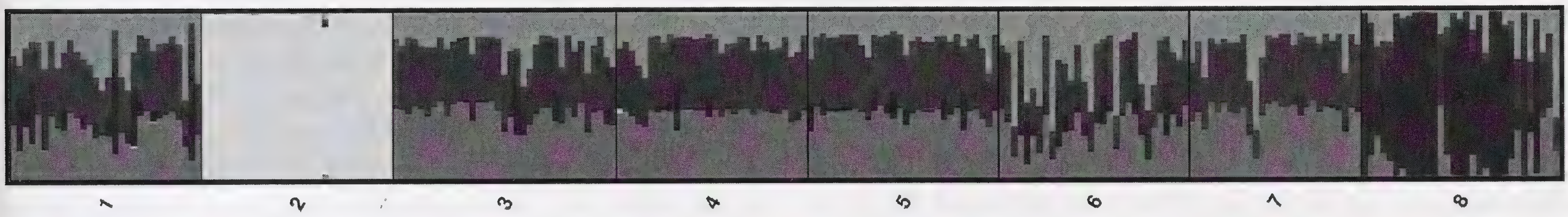

FIG. 2. STRUCTURE bar chart showing population composition. Numbers (x-axis) refer to populations in Table 1.

Genetic Diversity

The expected heterozygosity (H_e) value of Baker cypress (mean 0.204, SE 0.021), even when the anomalously low heterozygosity Flounce Rock population was included, was higher than reported values for two other cypress species, Tecate cypress (*Hesperocyparis forbesii* Jeps.) (mean 0.112, SE 0.019) (Truesdale and McClenaghan 1998), Monterey cypress (*H. macrocarpa* Hartw. ex Gordon) (mean 0.177, SE 0.035) (Conkle 1987), as well as Brewer spruce (*Picea breweriana* S. Watson) (mean 0.129, no SE given) (Ledig et al. 2005), another relictual species endemic to the Klamath region (Wolfe 1964). H_e for Baker cypress was also higher than knobcone, Bishop, and Monterey pines (*Pinus attenuata* Lemmon, *P. muricata* D. Don, and *P. radiata* D. Don), three species of fire-adapted, closed-

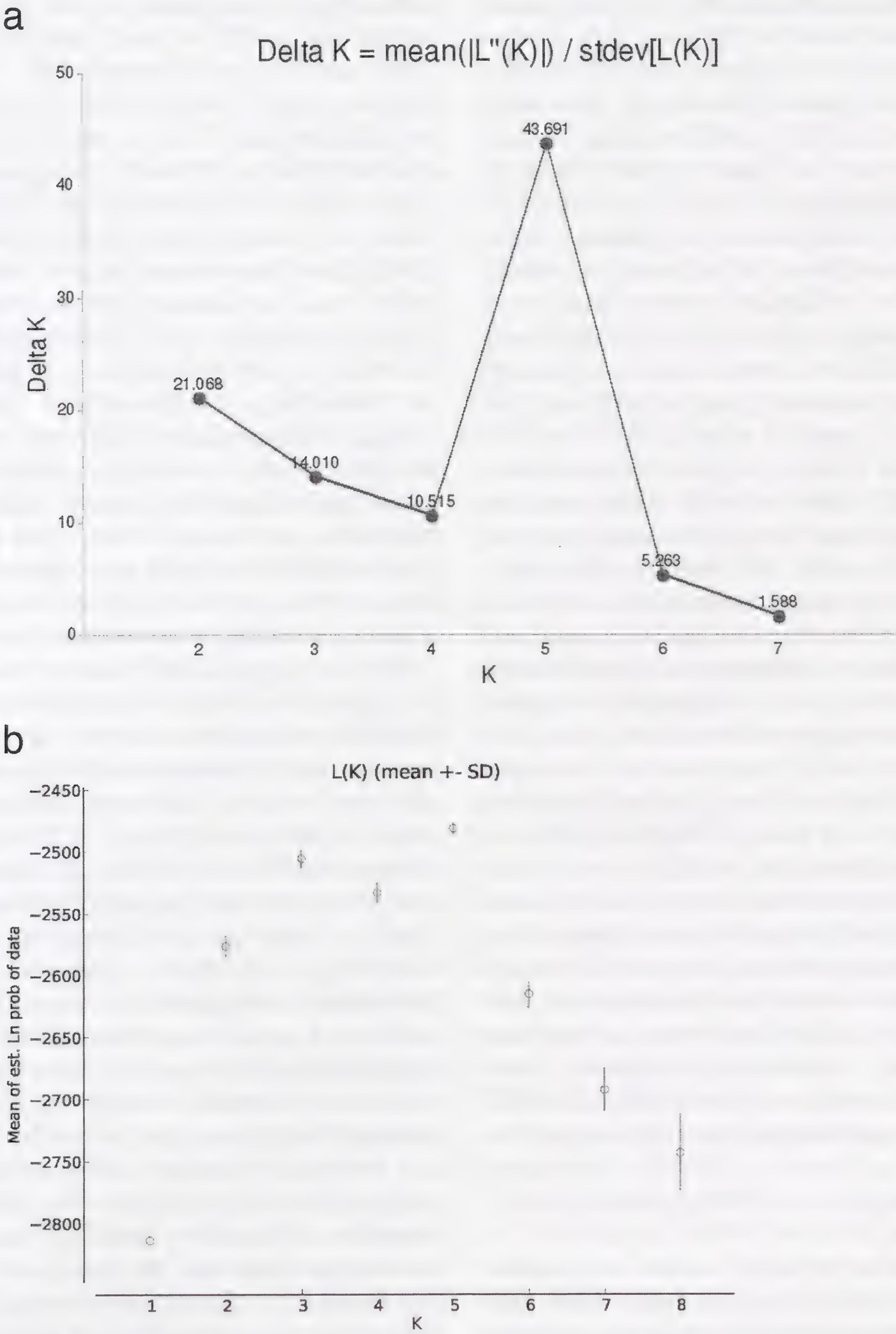

FIG. 3. A) Delta K and B) mean ln (K) from Evanno et al (2005) showing K = 5 indicating 5 distinct genetic groups.

TABLE 6. Wilcoxon sign-rank test for recent bottlenecks in eight Baker cypress populations under the infinite alleles model of mutation-drift equilibrium. a) number of polymorphic loci used in analysis, b) mean number of alleles, c) from Merriam and Rentz 2010.

Site	Loci[a]	Alleles[b]	Mean H_e	Prob.	Est. size (ha)[c]
Goosenest	8	1.79	0.233	0.014	120
Flounce Rock	5	1.36	0.106	0.078	1.2
Miller Lake	8	1.79	0.224	0.014	20
Timbered Crater	7	1.86	0.206	0.055	2800
Wheeler Peak	7	1.71	0.148	0.234	30
Burney	11	2.14	0.261	0.16	600
Seiad	7	2	0.215	0.148	320
Hamburg	9	1.86	0.265	0.005	6.4

cone (serotinous) pines also with restricted distributions in California (mean 0.138–0.160, no SE given) (Millar et al. 1988). Compared to other long-lived woody species, H_e for Baker cypress was considerably higher than for 115 outcrossing gymnosperms (0.169), 241 outcrossing species (0.180), 50 species with gravity dispersed seed (0.178) and 57 outcrossing endemics (0.142) (Hamrick and Godt 1996). The relatively high level of genetic diversity is surprising as most of the known extant populations are limited in size, some considerably so (Table 1), and it is likely that the current distribution of the species is a remnant of a more widespread distribution. These results may indicate that it is only relatively recently that the distribution of Baker cypress has been reduced and populations have become isolated, and there has not been sufficient time for genetic diversity to be lost due to random genetic drift and/or inbreeding. Another possible explanation for the relatively high genetic diversity is that the serotinous cones are maintained on the tree with viable seed for many years before the heat from a fire causes them to open and release their seeds. As a result, each yearly cohort of cones represents an independent sample of the pollen cloud and therefore the general gene pool of the stand. Depending on phenological and climatic differences from year to year, the genetic composition of the half-sib seeds produced each year will vary. Therefore, when all of these seed are released after a fire, the resulting seed bank that will give rise to the next generation of trees is likely to have a broader genetic base than if it was only seed that were produced in a single year as it would be for most non-serotinous conifers. This pattern was observed in the canopy seed bank of two long-lived woody shrubs with serotinous cone-like structures in fire-prone areas of Australia (*Banksia spinulosa* Sm. and *B. hookeriana* Meisn.). In both of these plants, over several years genetic variation in the aerial seed bank quickly approached the maximal level detected in the parental population and contained 100% of the adult genetic diversity (Ayre et al. 2010, Barrett et al. 2005).

Despite the relatively high levels of genetic diversity within the Baker cypress populations, many of the populations had fixation indices greater than zero (Table 3) indicating a deficiency of heterozygotes. A significant heterozygote deficiency was also reported in Tecate cypress by Truesdale and McClenaghan (1998), which they attributed to high levels of non-random mating (inbreeding) and a likely Wahlund effect. The most common explanation for heterozygote deficiency here is as a result of inbreeding, which is likely for Baker cypress as its seeds are wingless and gravity dispersed, usually following fires which cause the opening of the serotinous cones. This is likely to result in regeneration of familial "islands" composed of nearly even-aged groups of individuals related as half-sibs. When these trees reach reproductive maturity this could result in matings among related individuals resulting in the observed heterozygote deficiency. Since Baker cypress generally only reproduces after fires, it is possible that a Wahlund effect is also contributing to the heterozygote deficiency if periodic fires that stimulate regeneration only impact limited parts of the population, resulting in uneven aged pockets where age classes are closely related to fire history (Merriam and Rentz, 2010). This may be especially true in the Miller Lake population as the density of Baker cypress in this stand is low and the individuals sampled were dispersed over a fairly large area.

Of the populations tested, the Flounce Rock population exhibited the lowest values for all measures of genetic diversity: fewer average alleles per locus, and lower percent polymorphic loci as well as lower observed and expected heterozygosity than other populations (Table 3). This population is the northernmost extent of the species range, so there is a possibility that being a peripheral population, it may have had lower genetic diversity to begin with. It has been suggested effective population size and gene flow should be highest at the core of a species range and lowest at the range margins (Eckert et al. 2008). Relationships between heterozygosity and latitude have been reported in many species of forest trees in western North America (Jaramillo-Correa et al. 2006 and references therein) and have been attributed to postglacial recolonization patterns. Although a negative relationship between heterozygosity and latitude was detected in Brewers spruce, Ledig et al. (2005) argued against the post-glacial recolonization theory for this species as the pattern they observed was irregular and suggested random genetic drift in small populations rather than founder effects. They also argued that the postglacial recolonization theory is counter to the interpretation of Brewers spruce as a relic species that has persisted in the Klamath Region since the Miocene. The lack of any significant relationship between heterozygosity and latitude in Baker cypress similarly argues against the postglacial recolonization pattern and supports the idea that it also is a relictual species that was once more widespread.

Population Structure

Across all populations, Baker cypress showed moderate genetic structure. Genetic differentiation (F_{st}) (0.171) was very close to values reported for outcrossing species classified as having narrow ranges ($G_{st} = 0.169$) or endemic ($G_{st} = 0.179$), and for long-lived outcrossing species with gravity dispersed seed ($G_{st} = 0.177$), but higher than for outcrossing gymnosperms in general ($G_{st} = 0.073$) (Hamrick and Godt, 1992). However, due to the allele frequencies of the IDH locus, inclusion of the Flounce Rock population in the analysis had a strong influence on the overall level of population differentiation. With this population excluded, overall population differentiation was relatively low for such a highly fragmented species with geographically isolated populations (approximately 9% of the variation was among stands). This value was lower than both Monterey cypress (12% among stands) (Conkle 1987) and Tecate cypress (16% among stands) (Truesdale and McClenaghan, 1998), and also lower than the three California closed-cone pines (12, 22, and 13%) (Millar et al. 1988). The relatively low F_{st} (excluding Flounce Rock) for an endemic species with such a limited and disjunct distribution, and the lack of correlation between genetic and geographic distance suggests that the population structure in Baker cypress is due to random genetic drift in small, isolated populations. This may be further evidence that the extant populations of Baker cypress have only relatively recently contracted and become geographically isolated from each other. Raven and Axelrod (1978) propose that Tecate cypress was widespread during the Miocene and even into the Pliocene when summers were cooler and moister, colonizing serpentine areas as they became available. However, the widely distributed ecotypes disappeared as summer rains decreased during the early Pleistocene confining it to the serpentine areas as adaptation to the ultrabasic substrates removed them from competition from non-serpentine flora. In the more northern populations of its distribution, Baker cypress also occurs on serpentine soils, and in the southern populations it is often found on poorer volcanic soils. This supports the idea that the range contraction and population separation is of relatively recent origin. The current vegetation patterns likely developed only within the last 1500 to 3500 yr, and fossil evidence in general indicates a recent contraction of all cypress species north of Mexico, which have highly fragmented distributions and generally occur on soils inhospitable to other species (Callahan 2013). Species with limited geographic ranges often occur in small isolated populations which are prone to genetic drift resulting in reduced genetic diversity within populations and as a result of limited gene flow, increased genetic differentiation among populations (Hamrick et al. 1992). If this is the case with Baker cypress, it does not appear that genetic drift has had sufficient time to diverge the populations more; however, the results from the BOTTLENECK analysis suggest that a reduction in effective population size during range contraction may have resulted in a loss of allele numbers and heterozygosity at polymorphic loci in some populations. This loss of allelic diversity resulted in an observed heterozygosity larger than the heterozygosity that would be expected from the observed allele number if the locus was at mutation-drift equilibrium (Piry et al. 1999).

The genetic divergence of the Flounce Rock population was evident from both the pairwise F_{st} analysis and the STRUCTURE analysis. However, the STRUCTURE analysis also revealed one genetic cluster for the Hamburg population and three genetic clusters among the other populations (Fig. 2). The Hamburg and Seiad populations are the closest geographically, yet the pairwise F_{st} value between these populations (0.091, Table 4) is the largest among all populations (excluding Flounce Rock). The Hamburg population harbored a private allele for the MDH1 locus, however, reanalysis with this locus excluded still yielded $K = 5$ in a STRUCTURE analysis, and the pairwise F_{st} with the Seiad population, while slightly lower (0.072) was still one of the highest (excluding Flounce Rock). While there appears to be some clustering of these populations based on allele frequencies, the pairwise F_{st} analysis shows that these populations are all more closely related to each other than any of them are to Flounce Rock, by an order of magnitude (Table 4). The clusters determined by STRUCTURE do not fit a geographic pattern and as a result it is likely that the allele frequency differences among these populations are due to drift. In these small populations with limited, if any gene flow among populations, drift could result in different alleles moving towards fixation for various loci among populations. A higher resolution marker with more loci may be able to resolve this, so while the F_{st} values do indicate a some genetic differentiation, the results of the STRUCTURE analysis should be viewed with caution in regards to grouping genetically similar populations.

Due to the limited size (both geographically and in the number of individuals) of most Baker cypress populations, even a relatively small fire could kill all of the adult trees in a given population. Like most serotinous conifers, Baker cypress regenerates prolifically after fire, so a single fire likely would not pose a permanent threat to population persistence. However, if a subsequent fire kills all of the regeneration before it is able to reach reproductive age, this could lead to the extirpation of some of the smaller populations. These population genetic results show that there is genetic structure in the species most likely as a result of genetic drift. While some populations are genetically similar, there is no relationship between genetic and geographic distance, and therefore caution should be used if a decision is made to move seed among populations for purposes of reforestation.

CONCLUSIONS

Baker cypress is a rare, endemic tree species with small, highly isolated populations. Despite the high degree of geographic isolation among populations, levels of genetic diversity are higher than similar species made up of isolated populations. It has a moderate degree of genetic structure among populations, and most populations show a slight heterozygote deficiency, but our results indicate that the current population structure of the species is likely a fairly recent reduction from a formerly widespread distribution with drift leading to genetic differentiation. Baker cypress is a fire adapted species and it requires fire for regeneration. Recent fires in some stands have resulted in high levels of regeneration, however some of the smallest populations show significant impacts from fire exclusion, including high stand density, slow growth, and little or no regeneration. Introduction of fire to these stands is likely to be beneficial to these stands in the long run, but if regeneration fails for some reason, it could mean the catastrophic loss of an entire population. Seed collection and storage would be one form of insurance to prevent the loss of the genetic base of any population. The Moonlight Fire burned with a range of intensities through the Baker cypress population at Mud Lake, causing high mortality rates in some areas, but leaving other portions of the population (Merriam and Rentz 2010). While this resulted in substantial cypress regeneration after the fire (Merriam and Rentz 2010), a stand-replacing fire is not likely to be a suggested management recommendation for other small populations of Baker cypress that are currently in poor condition, such as the population at Flounce Rock. Actions to restore the condition of this, and other populations are likely to include treatments such as thinning, pile burning, and/or low intensity underburns, along with cone collections and planting seedlings (M. Wineteer, personal communication). Our results indicate that populations do differ genetically to some degree and these differences are not related to geography, so it is not advised to move seed among populations, even among ones that are geographically close. In addition, our results do not address potential differences in local adaptation, which could be assessed through a common garden test. It is recommended that seed be collected and used within the stands from which it originated and in populations most in need of restoration, activities be carefully planned to simulate natural burning to encourage natural regeneration, with the option to plant seedlings raised from the collected seed should natural regeneration fail. Thinning some of the denser areas of these stands, with some burning of both slash and duff may simulate a low intensity fire, providing the heat needed to open cones on the trees and release seeds and the bare mineral soil needed for these seeds to germinate.

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